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Supramolecular Chemistry

Saccharide-Containing Dynamic Proteoids

Yun Liu,^[a] Marc C. A. Stuart,^[a, b] Martin D. Witte,^[a] Eric Buhler,^{*[c]} and Anna K. Hirsch^{*[a, d, e]}

Abstract: Dynamic proteoids are dynamic covalent analogues of proteins, which can be used as new adaptive biomaterials. We designed and synthesized a range of sugar-containing dynamic proteoid biodynamers based on the polycondensation of different types of amino acid and dipeptide hydrazides with a biological aliphatic dialdehyde and a nonbiological aromatic dialdehyde. By using the saccharide-based dialdehyde, the biocompatibility of biodynamers should be enhanced compared to previously reported biodynamers.

Dynamic covalent chemistry (DCC)^[1] is a powerful tool to generate constitutionally dynamic analogues of biopolymers (also called biodynamers)^[2] at the molecular level, namely, molecular biodynamers.^[3] Molecular biodynamers can be produced by reversible covalent polymerization of biorelevant monomers. Therefore, their monomeric units are linked by reversible covalent bonds, such as oxime,^[4] acylhydrazone,^[5] disulfide,^[6] and

thioester bonds.^[7] As a consequence of the inherent nature of their bioactive constituents and reversible covalent linkages, molecular biodynamers possess both biorelevant character (i.e., biocompatibility, biodegradability, biofunctionality) and dynamic properties (i.e., changeable, tunable, controllable, self-healing, and stimuli-responsive capacities). Accordingly, the incorporation of nucleobase-, carbohydrate-, or amino acid-based monomers into molecular biodynamers through equilibrium polymerization leads to the generation of dynamic analogues of nucleic acids (DyNAs),^[8] polysaccharides (glycodynamers),^[9] or proteins (dynamic proteoids),^[10] respectively. In particular, the construction of dynamic proteoids gives rise to the fabrication of new adaptive and functional biomaterials, and may provide a new system to unravel the relationship between the specific 3D structure of a protein and its related biofunction.^[11] Hence, dynamic proteoids have attracted increasing research attention.

We have reported the preparation of dynamic proteoids with three different types of well-ordered nanostructures through reversible polycondensation of a water-soluble amphiphilic dialdehyde **1** with various bifunctional amino acid hydrazides (Scheme 1).^[10] We found that the polymerization is driven by the self-organization/folding of the dynamic proteoids formed through hydrophobic interactions. More importantly, side chains of amino acid hydrazides have a strong influence on the rates of polymerization, structures, and dynamic properties of the resulting dynamic proteoids, including the aromaticity, charge, and polarity.^[10b] Because the use of the nonbiological dialdehyde **1** leads to a decrease in biocompatibility of the resulting biodynamers, its replacement with a bio-based dialdehyde, such as a furanose-based dialdehyde **2** (Scheme 1)^[9] may circumvent this problem. On the other hand, the incorporation of saccharide residues and dipeptide hydrazides into dynamic proteoids not only gives new hybrids of biodynamers with potential biofunctionality and mimics of glycoproteins,^[12] but also offers further knowledge on the rational design and production of materials with desirable nanostructures. Based on these considerations, herein, we report the design and synthesis of a series of saccharide-containing dynamic proteoids through polycondensation of two dialdehydes (dialdehyde **1** is nonbiological and dialdehyde **2** is bio-derived) with various types of amino acid and dipeptide hydrazides bearing different side chains (Scheme 2).

Based on our previous study, we rely on the formation of reversible C=N bonds, including both imine and acylhydrazone bonds, to generate dynamic proteoids.^[10] The presence of two types of C=N bonds (true imines and acylhydrazones) affords biodynamers of double dynamicity and pH-responsiveness and potentially a third form of dynamic behavior through struc-

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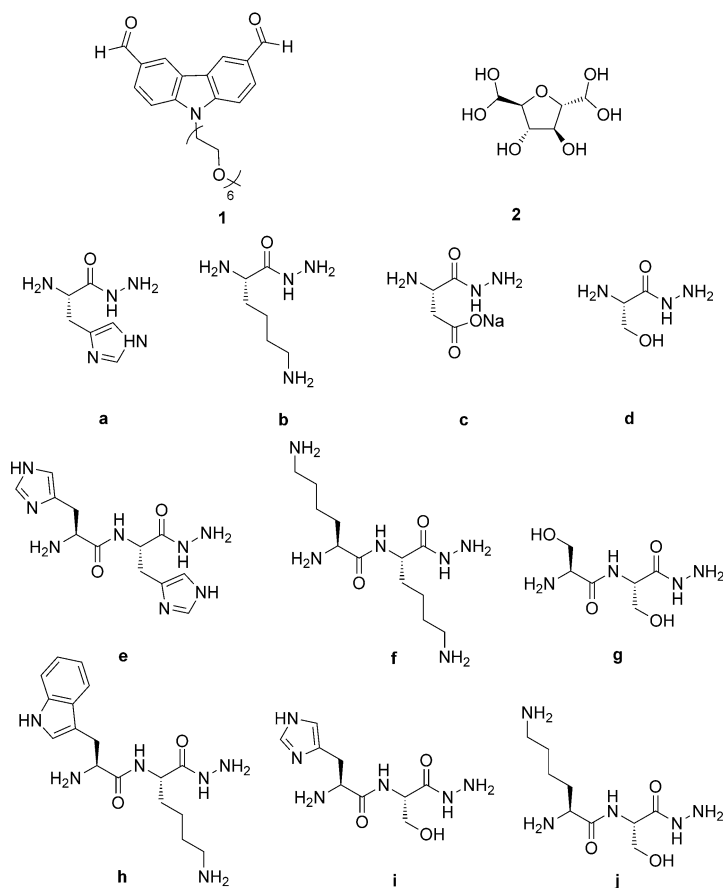
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Scheme 1. Structures of dialdehydes **1** and **2**, amino acid (**a–d**), and dipeptide hydrazides (**e–j**).

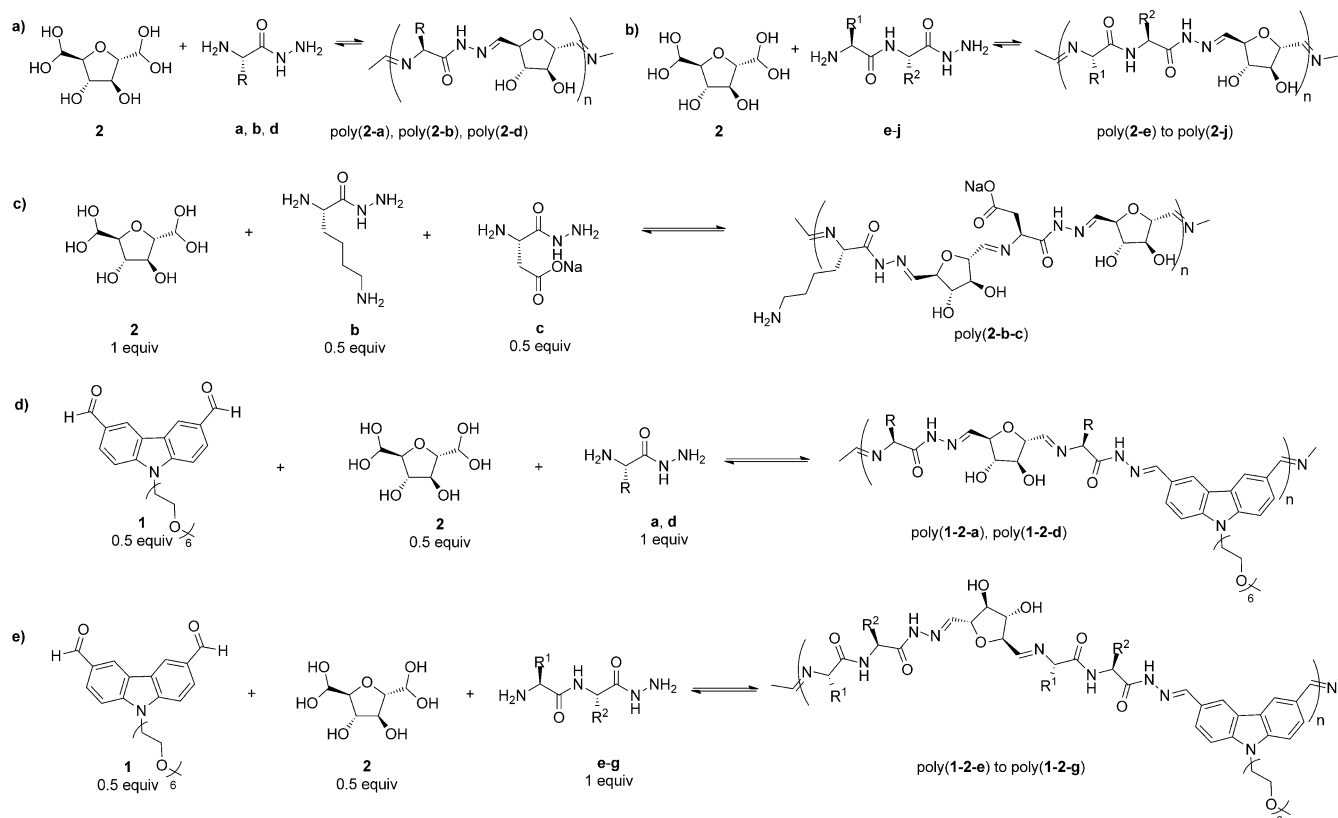
ture-formation processes (conformational dynamics). Through the polycondensation of dialdehydes with various amino acid or dipeptide hydrazides **a–j** (Scheme 1), dynamic proteoids with different particle sizes and stabilities are formed in aqueous media under mildly acidic conditions ($\text{pD} \approx 5$; Scheme 2).

We previously found the mechanism of polymerization to be nucleation elongation (NE),^[13] characterized by the formation of a critical size of polymer chain (nucleus) and elongation of the existing polymer, which is more favorable than initiation of a new chain. At $\text{pD} 5$, acylhydrazone formation proceeds readily and goes to completion, whereas the amine does not afford the corresponding imine. However, due to the hydrophobic and π – π -stacking interactions from the main chain, polymerization took place and generated thermodynamic biodynamers.^[10a] Our previous research demonstrates that three factors, namely, aromaticity, positive charge and hydroxyl groups, facilitate polymerization and stabilize the structures of biodynamers through non-covalent interactions between side chains of amino acid hydrazides and dialdehyde **1**, including π – π -stacking, cation– π interactions, and hydrogen bonds.^[10b] Furthermore, electrostatic forces dominate the polymerization when two oppositely charged amino acids are used. Along these lines, we designed and synthesized three categories of complementary dialdehyde monomers (Scheme 1): 1) amino acid hydrazides bearing an aromatic ring (**a**), a positive charge

(**b**), a negative charge (**c**), and a hydroxyl group (**d**); 2) dipeptide hydrazides containing two aromatic rings (**e**), two positive charges (**f**) or two hydroxyl groups (**g**), which should represent an enhancement of one beneficial factor; and 3) dipeptide hydrazides consisting of two different types of side chains, which represent a combination of two beneficial factors, such as an aromatic ring and a positive charge (**h**), an aromatic ring and a hydroxyl group (**i**), and a positive charge and a hydroxyl group (**j**). We synthesized monomers **1**, **2**, and **a–d** according to published procedures,^[9,10] and dipeptide hydrazides **e–j** by standard solid-phase peptide synthesis (SPPS; Scheme S1 in the Supporting Information). By following the polymerization through ^1H NMR spectroscopy and characterizing the biodynamers generated by dynamic light scattering (DLS), static light scattering (SLS), and cryo-transmission electron microscopy (cryo-TEM), we studied the respective influence of the three factors on polymerization and particle size of the resulting biodynamers, which could set the stage for the rational design of nanostructures as smart and functional biomaterials.

Replacement of dialdehyde **1** with a furanose-based dialdehyde **2** should improve the biocompatibility of the resulting biodynamers. Moreover, hydroxyl groups in dialdehyde **2** can stabilize the biodynamers through hydrogen bonds and/or OH– π interactions. Additionally, by comparing the aromatic dialdehyde **1** with the aliphatic dialdehyde **2**, the influence of the aromatic backbone on biodynamer formation can be evaluated. Polycondensation of dialdehyde **2** with monomeric hydrazides **a–j** in mildly acidic aqueous $[\text{D}_3]\text{acetate}$ buffer at room temperature gave the corresponding biodynamers, which we monitored by ^1H -NMR spectroscopy (Figure S1 in the Supporting Information). The consumption of dialdehyde **2** (Table S1 in the Supporting Information) illustrates that only poly(**2–a**), poly(**2–e**), and poly(**2–h**) formed and consumed most of dialdehyde **2** at room temperature, whereas the other hydrazides only consumed a small amount of dialdehyde **2** at equilibrium. Such findings can be attributed to the decreased reactivity of dialdehyde **2** compared with dialdehyde **1**. However, it also demonstrates the importance of aromatic main chains for the formation of biodynamers. An aromatic core provides the driving force for polymerization and stabilizes the resulting dynamic proteoids through π – π -stacking interactions. On the other hand, the successful generation of poly(**2–a**), poly(**2–e**), and poly(**2–h**) at room temperature also demonstrates the importance of aromatic side chains in polycondensation through π – π stacking interactions. To generate the remaining biodynamers, we envisaged to enhance the extent of polymerization by increasing the reaction temperature or adding dialdehyde **1**.

Because imine formation can be accelerated by raising the reaction temperature,^[14] we performed the polycondensations at a higher temperature (50°C ; Figure S2 in the Supporting Information) and observed significant increments in consump-



Scheme 2. Schematic representation of the preparation of dynamic proteoids through reversible polycondensation of (a) dialdehyde **2** with amino acid hydrazides **a**, **b**, **d**; (b) dialdehydes **2** with dipeptide hydrazides **e-j**; (c) dialdehydes **2** (1.0 equiv) with amino acid hydrazides **b** (0.5 equiv) and **c** (0.5 equiv); (d) dialdehydes **1** (0.5 equiv) and **2** (0.5 equiv) with amino acid hydrazides **a** or **d** (1.0 equiv); (e) dialdehydes **1** (0.5 equiv) and **2** (0.5 equiv) with dipeptide hydrazides **e-g** (1.0 equiv) in aqueous [D₃]acetate buffer at pH 5.

tion of dialdehyde **2** in all cases (Table S1 in the Supporting Information). This leads to the formation of the corresponding biodynamers and demonstrates that higher temperature speeds up polycondensation and facilitates the generation of biodynamers. Poly(2-b-c) was designed bearing oppositely charged side chains to evaluate the importance of electrostatic interactions. Because poly(2-b-c) cannot form at room temperature, it demonstrates that electrostatic attractions between oppositely charged monomers are not as strong a driving force as aromatic rings to initiate polycondensation. Compared with poly(2-d) and poly(2-b) at 50 °C, however, the extent of polymerization of poly(2-b-c) was increased by the electrostatic attractions. We characterized the particle sizes and aggregation number of the dynamic proteoids formed by DLS, SLS (Table S3, Figures S6 and S7 in the Supporting Information) and cryo-TEM (Figures S3 and S5 in the Supporting Information). We observed globular objects with sizes ranging between approximately 1 and 5 nm. For solutions composed of particles with hydrodynamic radius R_h close to 1 nm, the DLS signal is rather low giving a larger error bar for R_h and molecular weight. For some samples, the correlation function is bimodal indicating the presence of a minority second population of larger aggregates, which can be neglected (see the Supporting Information). Poly(2-e) has one of the biggest particle sizes, presumably due to π - π stacking interactions. Additionally,

poly(2-g) bearing hydroxyl groups is stabilized through hydrogen-bonding interactions.

We prepared poly(1-2-a), poly(1-2-d), poly(1-2-e), poly(1-2-f), and poly(1-2-g) through polymerization of half an equivalent of both dialdehydes **1** and **2** with one equivalent of the corresponding amino acid hydrazide at room temperature (Scheme 2c and d; and Figure S4, Table S2 in the Supporting Information). In the case of poly(1-2-d) and poly(1-2-g), 51 and 53% of dialdehyde **2** was consumed in comparison with 4 and 26% for poly(2-d) and poly(2-g), respectively (Table S2 in the Supporting Information). We observed no clear enhancement in product formation in the case of poly(1-2-a), poly(1-2-e), and poly(1-2-f). In some cases, addition of dialdehyde **1** may stabilize the generated biodymer through π - π stacking and/or OH- π interactions and result in significant enhancement of product yield. From LS and the cryo-TEM image of poly(1-2-a) (Table 1 and Figure S3e in the Supporting Information), we observed globular nano-object structures with a particle size of 1.42 ± 0.17 nm, which can be ascribed to π - π stacking interactions between the aromatic cores of dialdehyde **1** and the aromatic side chains of monomer **a**. In addition, hydroxyl groups in dialdehyde **2** may also contribute to the resulting architectures through hydrogen-bonding and OH- π interactions. Moreover, poly(1-2-d), poly(1-2-e), and poly(1-2-g) are much larger nanostructures with higher aggre-

Table 1. Structural parameters obtained from cryo-TEM and light scattering (LS).

Sample	R [nm] ^[a]	R_h [nm] ^[b]	R_g [nm] ^[c]	M_{dimer} [g mol ⁻¹]	$M_{\text{w,app}}$ [g mol ⁻¹]	N
poly(2-a)	0.96 ± 0.16	1.20 ± 0.2	–	329.4	4525	14
poly(2-b)	1.26 ± 0.12	1.48 ± 0.3	–	320.4	4716	15
poly(2-d)	1.74 ± 0.13	– ^[d]	–	279.3	5150	18
poly(2-b-c)	0.85 ± 0.09	1.25 ± 0.3	–	649.7 ^[e]	6574	10
poly(2-e)	5.10 ± 0.39	2.1 ± 0.8	–	466.5	27 188	58 ^[f]
poly(2-f)	0.76 ± 0.10	0.8 ± 0.2	–	448.6	22249	50 ^[f]
poly(2-g)	3.54 ± 0.22	– ^[g]	–	366.4	– ^[g]	– ^[g]
poly(2-h)	1.55 ± 0.19	4.4 ± 0.8	–	506.6	36220	71 ^[f]
poly(2-i)	1.38 ± 0.12	– ^[d]	–	416.5	3244	8
poly(2-j)	1.43 ± 0.15	1.1 ± 0.3	–	407.5	15295	38 ^[f]
poly(1-2-a)	1.42 ± 0.17	2.3 ± 1	–	980.2 ^[e]	18203	19
poly(1-2-d)	– ^[g]	27.4 ± 1	64 ± 5	880.0 ^[e]	492996	560
poly(1-2-e)	– ^[g]	23.6 ± 1	54 ± 5	1254.4 ^[e]	568220	453
poly(1-2-f)	– ^[g]	– ^[d]	– ^[d]	1218.6 ^[e]	– ^[d]	– ^[d]
poly(1-2-g)	– ^[g]	23.1 ± 1	62.9 ± 5	1054.2 ^[e]	684398	649

[a] Particle radius obtained from cryo-TEM experiments. [b] R_h = apparent hydrodynamic radius obtained from DLS measurements. [c] R_g = radius of gyration obtained from SLS measurements for particles larger than 20 nm. [d] Signal was too low. [e] M_w of tetramer. [f] The presence of a slow mode associated to a minority population of large aggregates overestimates the calculated aggregation number of the nano-objects. [g] Experiments were not performed. For the other solutions error bar is $\approx 10\%$. M_{dimer} = dimer molecular weight; $M_{\text{w,app}}$ = apparent weight-averaged molecular weight obtained from SLS; and N = aggregation number.

gation numbers than corresponding poly(2-d), poly(2-e), and poly(2-g), which demonstrates that the addition of dialdehyde 1 provides further stability to the resulting biodynamers and facilitates the formation of nanostructures through π - π -stacking interactions. Their R_g/R_h ratio is larger than 2, indicating the formation of elongated structures, such as rods, and already observed for similar systems.^[10b, 15] Assuming a rod-like structure, an estimate of the length of the rods of approximately 200 nm can be obtained by using the radius of gyration: $L = R_g\sqrt{12}$. In addition, the rate of polymerization (poly(1-2-a)) was studied by monitoring the consumption of dialdehyde 1 and 2 in polycondensation (Figure S8 in the Supporting Information). At the very beginning of the polymerization, dialdehyde 2 was consumed much faster than dialdehyde 1, which demonstrates that dialdehyde 2 was quickly involved into the nucleation process to generate the nucleus for the polymerization owing to its weak steric hindrance, and a small percentage of dialdehyde 1 could easily stabilize the nucleus to initiate the polymerization. When the polymerization proceeds, more and more dialdehyde 1 is incorporated into the resulting polymers to drive the polymerization and stabilize the dynamic proteoids formed. Taken together, biodynamers with improved biocompatibility and well-ordered structures can be generated through both methods, which are stabilized by self-folding of the resulting polymers through various non-covalent interactions, including π - π -stacking, hydrogen-bonding, and OH- π interactions.

In conclusion, we designed and prepared a range of saccharide-containing dynamic proteoids through formation of two types of reversible C=N bonds (imine and acylhydrazone). To improve the biocompatibility, we incorporated biological dialdehyde 2 into the resulting biodynamers. The use of sugar-derived dialdehyde 2, however, decreased the extent of polymerization. Thus, we improved the extent of the reaction between

hydrazides with dialdehyde 2 by raising the reaction temperature or adding aromatic dialdehyde 1. We characterized polymerization and the particle size of the biodynamers formed and demonstrated that the nature of the side chains of amino acid hydrazides, including their aromaticity, charge and polarity, have a strong influence on polymerization and particle size of the resulting biodynamers. Taken together, these findings provide a combination of chemical, biological, and combinatorial approaches to design and prepare dynamic analogues of proteins (glycoproteins). The biodynamers generated combine the biocompatibility and functionality of biological components with adaptability stemming from the dynamic covalent bonds to achieve synergistic properties, which can be used as adaptive functional biomaterials in both biomedical and bioengineering fields. More specifically, the construction of dynamic proteoids might enable the design and production of glycoproteins with new or desired functions by unravelling the relationship between 3D structure and biofunction. In addition, protein-protein complexes are an emerging class of drug targets. The use of sugar-containing dynamic proteoids might also offer a new strategy to design, identify, and synthesize dynamic inhibitors of protein-protein interactions^[16] to overcome their large, flat, hydrophobic, and solvent-exposed contact surfaces.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biodynamers • dynamic proteoids • equilibrium polymerization • nanostructures • supramolecular chemistry

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